Prenatal Malnutrition-Induced Functional Alterations in Callosal Connections and in Interhemispheric Asymmetry in Rats Are Prevented by Reduction of Noradrenaline Synthesis during Gestation¹,²,³

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ABSTRACT  Prenatal malnutrition results in increased concentration and release of central noradrenaline, a neurotransmitter that is an important regulator of normal regressive events such as axonal pruning and synaptic elimination. This suggests that some of the functional disturbances in brain induced by prenatal malnutrition could be due at least in part to increased noradrenaline activity that may enhance regressive events during early stages of development. To test this hypothesis we studied whether chronic administration of α-methyl-p-tyrosine, an inhibitor of tyrosine hydroxylase, to rats during gestation might prevent long-term deleterious effects of prenatal malnutrition on functional properties of interhemispheric connections of the visual cortex, and on asymmetry of visual evoked responses. The experiments were conducted on normal and malnourished rats 45–50 d of age. Prenatal malnutrition was induced by restricting the food consumption of pregnant rats to 40%, from d 8 postconception to parturition. At birth, prenatally malnourished rats had significantly greater whole-brain noradrenaline concentration as well as significantly enhanced noradrenaline release in the visual cortex. At 45–50 d of age, the malnourished group had a significantly smaller cortical area, exhibiting transcallosal evoked responses; in addition, the amplitude of these responses was significantly smaller. Malnourished rats showed a significant reduction of the normal interhemispheric asymmetry of visual evoked responses. The addition of 0.3% α-methyl-p-tyrosine to the diet of malnourished pregnant rats during the last 2 wk of gestation prevented functional disorders induced in the offspring by prenatal malnutrition on interhemispheric connectivity of visual areas and on interhemispheric bioelectric asymmetry, probably by reducing the elevated brain noradrenaline activity and thereby restoring the normal trophic role of this neurotransmitter.  J. Nutr. 128: 1224–1231, 1998

KEY WORDS:  • prenatal malnutrition  • brain development  • noradrenaline synthesis  • rats  • callosal and visual responses

A great body of evidence indicates that prenatal malnutrition can induce morpho-functional alterations in the brain that could underlie long-lasting behavioral disorders (reviewed in Levitsky and Strupp 1995, Morgane et al. 1993. As has been pointed out in the literature (Morgane et al. 1993), prenatal malnutrition could alter brain growth and development by affecting a variety of cellular processes, for example, by reducing the number of cells, by perturbing and desynchronizing cellular migration, by delaying or blocking cellular growth and differentiation and by increasing cellular death. It is important to note that those cellular processes that are affected by prenatal malnutrition are profoundly influenced by metabolic properties of the milieu under normal conditions. In fact, before synapse formation, monoamines may exert a paracrine role because they could serve as chemical signals participating in the regulation of cellular events during early brain development such as neurogenesis, migration of neurons, differentiation and maturation of neurons, synaptogenesis, and even regression and programmed cellular death (Huether 1989, Morgane et al. 1993). On those bases, the issue that nutritional inadequacies during prenatal life increase central noradrenaline (NA)⁵ levels (Resnick and Morgane 1983) and enhance central NA release (Soto-Moyano et al. 1994) is of particular relevance, considering that the normal effect of noradrenergic innervation is of an inhibitory nature, limiting the formation


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⁵ Abbreviations used: aMT, α-methyl-p-tyrosine; KRB, Krebs-Ringer bicarbonate; NA, noradrenaline; TER, transcallosal evoked responses; VER, visual evoked responses.
of connections during brain development (Maeda et al. 1974, Parnavelas and Blue 1982, Wendlandt et al. 1977). It is likely that this noradrenergic regulatory mechanism starts very early, because of the following conditions in rats: 1) the cells that form the locus coeruleus undergo a period of intense mitotic activity between 11 and 13 d of gestation, after which cell division ceases (Johnston et al. 1979); 2) neurons storing NA are detectable in the brain as early as the d 12–13 of embryonic life (Hamon and Bourgoin 1981); and 3) tyrosine-hydroxylase and dopamine-β-hydroxylase activities, indices of functional maturation of noradrenergic neurons, have been demonstrated in the brain of fetal rats at 15 d of gestation (Coyle and Axelrod 1972a and 1972b). From these studies, it is apparent that the noradrenergic system in rats is “operative” by the end of gestation and could therefore, be influenced as early as during fetal life by environmental and nutritional stressors.

Because prenatal malnutrition increases central NA activity and NA participates in the early, normal development of the brain, some of the modifications induced by nutritional injuries on brain structures could be due at least in part to the high activity of central NA, which in turn can disrupt normal brain cellular events. If so, pharmacological reduction of the enhanced central NA activity during development should prevent some of the brain disorders induced by prenatal malnutrition.

This study was designed to investigate in rats whether chronic administration during gestation of α-methyl-p-tyrosine (αMT), an inhibitor of tyrosine hydroxylase, might prevent long-term deleterious effects of prenatal malnutrition on functional properties of interhemispheric connections of the visual cortex and on asymmetry of visual evoked responses. As has previously been reported (Soto-Moyano et al. 1993), in utero malnutrition in rats alters both the functional pattern of transcallosal evoked responses (TER) and the normal asymmetry of visual evoked responses (VER). It seems important to stress that interhemispheric visual cortical connections travel mainly through the corpus callosum, a structure that has been shown to play key roles in integrating the motor, sensory and cognitive experience of the two sides of the brain (Geschwind and Galaburda 1985). On the other hand, brain asymmetries in rats are parallel to those found in humans and probably are subserved by similar neurobiological mechanisms (Glick and Ross 1981).

MATERIALS AND METHODS

The experimental protocol and animal management were in accordance with the NIH guidelines (NRC 1985). The experiments were conducted on male and female Wistar rats (INTA, Santiago, Chile) born from dams subjected during pregnancy to one of the following nutritional conditions: 1) well-nourished pregnant rats, with free access to a 21% protein nonpurified diet (Champion, Santiago, Chile); 2) malnourished pregnant rats, with free access to food until d 7 postconception; after this date the nonpurified diet was restricted to 10 g/d until parturition. This amount of food is ~40% of that consumed by normal pregnant rats during wk 2 and 3 of gestation (Soto-Moyano et al. 1993). Day 8 postconception was selected to initiate food restriction, because reduced indices of reproduction have been shown in rats when this paradigm of protein-energy deprivation is introduced from d 2 postconception (Chow and Lee 1964). This could be attributable to the fact that implantation of the fertilized ovum occurs at the end of d 5 postconception in rats (Wimsatt 1975). At birth, to ensure adequate nutrition during lactation, prenatally malnourished pups were fostered to well-nourished dams giving birth on that day.

During the suckling period, all litters were adjusted to 8 pups per dam; at weaning, the offspring were given free access to the nonpurified diet. The body weight of dams was determined during pregnancy. The body and brain weights of pups were determined at birth and at the age of experiments. Brain weight measurements were performed excluding cerebellum, brainstem and olfactory bulbs.

According to the pharmacological treatment imposed on the dams during pregnancy, the rats were grouped as follows: 1) normal rats, born from dams with free access to nonpurified diet throughout pregnancy; 2) normal-αMT rats, born from dams with free access to nonpurified diet containing 0.3% of αMT during the last 2 wk of pregnancy; 3) malnourished rats, born from dams receiving restricted (10 g) nonpurified diet during the last 2 wk of pregnancy; 4) malnourished-αMT rats, born from dams receiving restricted nonpurified diet containing 0.3% of αMT during the last 2 wk of pregnancy. The drug was added to the nonpurified diet because it has been reported that oral administration of αMT results in a reduction of brain NA synthesis comparable to that of subcutaneous or intraperitoneal injections (Johnson et al. 1967).

Neurochemistry. Whole-brain and visual cortex NA concentrations were assayed on d 1 and 45 of postnatal life. The rats were killed by decapitation and the brains were rapidly removed and cooled on ice. The tissue was treated with perchloric acid (0.4 mol/L) containing sodium bicarbonate and sodium fluoride and stored at 4°C until analyzed. The tissue was then disrupted by homogenization and 0.1 mL of homogenate was used for protein determination. Alumina was added to the remaining tissue to absorb the catecholamines, and the samples were washed and eluted in perchloric acid 0.1 mol/L with the use of a microfilter kit. Aliquots of the eluted extract were subjected to HPLC. For chromatographic separations, a Biophase ODS (5 μm) analytical column was used (Bioanalytical System, West Lafayette, IN). The mobile phase was a mixture of 0.15 mol/L monochloroacetate, pH 3.0, with 2.0 mmol/L Na2 EDTA and 28 mg/L sodium octyl sulfate. All solutions were filtered and degassed. The 20-μL injection was made through a fixed loop injection valve at flow rates of 1.3–1.5 mL/min.

Occipital cortex NA release was determined on d 1 and 45 of postnatal life. After the brain was removed, the occipital pole was dissected and cortical slices of 240 μm were obtained using a tissue slicer by chopping in one direction. The slices were preincubated for 10–15 min at 37°C in 2.0 min of Krebs-Ringer bicarbonate (KRB) buffer according to a method reported elsewhere (Gothert et al. 1979). Eight to ten slices were incubated for 50 min at pH 7.4 in 1.0 mL of KRB saturated with 5% CO2 in O2 containing 0.111 MBq of 3H-NA and thereafter transferred to a superfusion chamber. Slices were submitted to three cleaning periods of 20 min each followed by another one of 10 min. They were then subjected to four periods of basal release of 2 min each followed by a 4-min stimulation period in which the perfusion solution was removed and changed by a solution containing K+ 30 mmol/L. Afterward, normal KRB was replaced and superfusion samples were collected during six periods of 2 min each. During the periods of cleaning, basal release and potassium-stimulated release of 3H-NA, a constant flux rate of 1.0 mL/min was maintained by using a peristaltic pump. At the end of the experiments, the slices were homogenized in 0.92 mol/L of trichloroacetic acid. Total homogenates were centrifuged at 12,500 × g for 20 min, and the radioactivity present in the supernatant and in the superfusion fractions collected was measured by liquid scintillation counting. Counting efficiency was 35% in a Nuclear Chicago Scintillation Counter (Chicago, IL). The scintillation mixture used to count radioactivity was PPO (4 g/L); POPOP (0.1 g/L) dissolved in toluene. Basal (spontaneous) as well as potassium-induced release of radioactivity was calculated for each fraction as a percentage of fractional release (percentage of radioactivity released in relation to the radioactivity content of the tissue at the time of collection of samples). Results were expressed as net percentual fractional release (induced release minus basal release).

Electrophysiology. At 45–50 d of age, normal, normal-αMT, malnourished and malnourished-αMT rats were anesthetized with 100 mg/kg of α-chloralose and placed in a stereotaxic apparatus. A single dose of 1.5 mg/kg of d-tubocurarine was injected intramuscu-
larly as a muscle relaxant, and adequate ventilation was maintained by means of a respiratory pump. Because cortical responses were led in a differential amplifier, d-tubocurarine was used to avoid muscle responses recorded through the reference electrode. This electrode was located over cotton soaked in saline placed in the midline of the frontal bone, but in contact with the excised muscles to ensure good electrical conductivity. Reinforcement of anesthesia during the experiments was not necessary because surgical procedures and recordings lasted no longer than 1.5 h. In our experience with nonparalyzed rats, the dose of α-chloralose used produces profound anesthesia lasting 2 h. Moreover, no changes in heart rate in response to stimulation were detected throughout the experiments.

TER were studied in normal (n = 11), normal-αMT (n = 8), malnourished (n = 10) and malnourished-αMT (n = 11) rats. After exposure of the occipital lobe of both cerebral hemispheres, electrical stimulation of the left primary visual area was carried out by means of a bipolar electrode placed at the de Groot coordinates: anteriority = 0.0, laterality = 3.5, in mm. Cortical electrical stimulation consisted of single rectangular pulses of 25 μA strength, 0.5 ms duration and 0.25 Hz frequency. TER were recorded from the right visual cortex, every 0.5 mm, with a monopolar silver ball electrode. The de Groot’s coordinates of the cortical surface explored were the following: anteriority = from 5.0 to −2.0; laterality = from 0.5 to 5.0, in mm. Recordings of TER were amplified (0.8–1000 Hz: bandwidth), displayed on an oscilloscope and digitized at a rate of 10,000/s by an analogical-digital converter interfaced to a microcomputer. They were also stored on hard disk for retrieval and averaging. The mean amplitude of all responses recorded in the explored region was calculated, and only those responses that exceeded the mean by 1.5 SD were considered in this study. These responses were designated as high energy TER. Body temperature and expired CO2 were monitored and remained within normal limits throughout the experiments.

The interhemispheric asymmetry of VER was studied in other normal (n = 14), normal-αMT (n = 9), malnourished (n = 13) and malnourished-αMT (n = 13) rats. After bilateral exposure of the occipital cortex, binocular photic stimulation was carried out by means of flashes of white light (5 lumens/m²: intensity, 1 ms duration, 0.125 Hz frequency). The lamp of the photic stimulator was placed in the median of the sagittal plane of the rat, 30° above the horizon. A dim background illumination was maintained throughout the experiments. Pupillary motility was controlled by topical corneal application of a 10 g/L solution of atropine sulfate. VER were simultaneously recorded from both the right and the left primary visual areas with monopolar silver ball electrodes placed at the following de Groot’s coordinates: anteriority = 0.0, laterality = 4.0, in mm. As in the case of TER, VER were amplified, displayed on an oscilloscope, digitized by an analogical-digital converter interfaced to a microcomputer and stored on hard disk for retrieval and averaging. Results were expressed as difference in peak-to-peak amplitude of VER recorded from the dominant and the non-dominant hemisphere. Dominant hemisphere was defined as that exhibiting responses of higher peak-to-peak amplitude than those exhibited by the other one.

At the end of the electrophysiologic experiments, the rats were killed by an intraperitoneal overdose of sodium pentobarbital.

Statistical analysis. Data are reported as means ± SEM. Two-way ANOVA was used to determine the effect of diet or αMT and their interaction on all of the variables studied. When a P-value in the ANOVA was <0.05, Newman-Keuls test was used for multiple comparisons (Rosner 1990).

RESULTS

Weight gain of the dams. From d 6–13 of pregnancy, no significant differences in body weight were observed among the four groups studied. From d 15 to parturition, mean body weight of normal dams with or without αMT treatment was significantly higher than those of both groups of malnourished dams. Statistical analysis shows that there was no significant interaction between diet and αMT during pregnancy (Table 1).

Body and brain weights of pups. At birth, malnourished pups exhibited a significant body weight deficit compared with normal pups. αMT treatment did not modify the birth weight of normal and malnourished pups. At 45 d of age, no differences in body weight were observed among the four groups. At these two ages, no interaction was observed between dietary and αMT treatments (Table 2). At d 1 of age, malnourished pups showed a significant brain weight deficit compared with normal pups. αMT treatment improved the brain weight of malnourished pups but did not modify the brain weight of normal pups. The statistical tests suggest that αMT interacted significantly with diet in improving brain weight of malnourished pups. At 45–50 d of age, brain weight deficits were observed in both malnourished and malnourished-αMT rats. At this later age, no diet × αMT interaction was observed (Table 2).

Noradrenaline determinations. Determinations of whole-brain NA concentration showed that at d 1 of postnatal life, malnourished pups had higher NA levels than normal pups. Administration of αMT to normal and malnourished pregnant dams resulted in lower NA concentration in the brain of the newborns. Statistical analysis shows that at this age malnourished-αMT pups exhibited values of whole-brain NA concentration similar to those of normal pups and that there was no interaction between diet and αMT (Table 3). At d 45 of postnatal life, no significant differences in whole-brain NA concentration were observed among normal, malnourished and malnourished-αMT rats. In contrast, normal-αMT rats had a significant deficit in this variable. At this age, a significant interaction diet × αMT was observed (Table 3).

Assays of visual cortex NA concentration performed at d 1 of postnatal life showed a trend similar to that observed for whole-brain determinations, although the difference between normal and malnourished groups did not reach significance. At d 45 of age, no significant differences in visual cortex NA concentration were observed among the four groups of rats. Statistical analysis showed that at both ages no significant interactions between diet and αMT occurred (Table 3).

Determinations of visual cortex NA release showed that at d 1 of postnatal life, malnourished pups had higher NA release than normal pups. Administration of αMT to normal and malnourished pregnant dams resulted in lower cortical NA release in the brain of the newborns. After αMT treatment, malnourished pups exhibited values of visual cortex NA release similar to those of normal pups (Table 3). At d 45 of age, no significant differences in visual cortex NA release were observed among normal, malnourished and malnourished-αMT rats. In contrast, normal-αMT rats exhibited a significantly higher cortical NA release. At these two ages, no interaction was observed between dietary and αMT treatments (Table 3).

Transcallosal evoked responses. At 45–50 d of age, the extent of the area occupied by high energy TER was markedly reduced in the malnourished group compared with the normal group (Fig. 1). Prenatal αMT resulted in a greater high energy TER area in malnourished rats but did not modify the extent of this area in normal rats. The statistical analysis suggests that αMT interacted significantly with diet in the effect observed. A similar trend was observed for TER amplitude. (Fig. 1).

Interhemispheric asymmetry of visually evoked responses. At 45–50 d of age, normal and normal-αMT rats had significant differences in peak-to-peak amplitude of VER recorded from symmetrical points in the two cerebral hemispheres (Fig. 2). This asymmetry was reduced in malnourished rats of the same ages. In contrast, malnourished-αMT rats exhibited asymmetries in peak-to-peak amplitude of VER that did not differ from those observed in normal rats. No interaction between diet and αMT was observed (Fig. 2).

DISCUSSION

Reduction of food intake during pregnancy resulted in a lower maternal weight gain. Administration of αMT to either
normal or malnourished dams did not modify the evolution of weight gain during pregnancy. It has been argued that αMT induces sedation as a result of central catecholamine depletion (Seiden et al. 1975), which could lead to energy saving and higher weight gain of rats as a result of decreased locomotor activity. This could explain the fact that at d 21 of pregnancy, normal-αMT rats were slightly heavier than normal rats. This effect was not observed in malnourished rats receiving αMT, probably because of the intense locomotor activity exhibited by rats subjected to food restriction (Shibata et al. 1994).

Malnutrition during gestation resulted in significant body weight deficit of pups at birth, indicating that reduction of food intake by the dams during pregnancy caused fetal growth retardation. This effect was observed in both the malnourished and the malnourished-αMT groups, revealing that the pharmacological treatment was unable to reverse prenatal malnutrition-induced body weight deficit at birth. Fostering of the pups to well-nourished dams giving birth on that day led to body weight recovery of the offspring. Similar results have been obtained by others (Smart et al. 1973).

Malnutrition during gestation also resulted in a significant brain weight deficit in newborns. This effect was partially prevented by the αMT treatment, as indicated by the significantly higher brain weights in the malnourished treated group compared with the malnourished untreated group, albeit malnourished-αMT pups did not reach the brain weight values of normal newborns. On the bases that nutritional inadequacies during prenatal life increase central NA activity (Resnick and Morgane 1983, Soto-Moyano et al. 1994) and that NA exerts early inhibitory trophic influences on normal brain development (Caviness 1989), it is plausible that in this study, reduction of the enhanced central NA availability by αMT could have led to a suitable amount of NA released in the milieu during early maturation of the brain, resulting in a partial preventive effect on prenatal malnutrition-induced brain weight deficit. αMT administration to normal pregnant rats did not affect either the body weight or the brain weight of pups at birth. Comparable results have been previously reported by others (Rosengarten and Friedhoff 1979). Brain weight measurements performed at 45 d revealed a significant brain weight deficit in both the malnourished and the malnourished-αMT groups. As has been pointed out in the litera-

### Table 1

**Effect of malnutrition and α-methyl-p-tyrosine (αMT) administration on body weights of female rats during pregnancy**

<table>
<thead>
<tr>
<th>Pregnancy day</th>
<th>Normal</th>
<th>Normal αMT</th>
<th>Malnourished</th>
<th>Malnourished αMT</th>
<th>2-way ANOVA</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Diet</td>
<td>αMT</td>
<td>Diet</td>
<td>αMT</td>
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<td></td>
<td>P</td>
<td>P</td>
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<td>6</td>
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</tr>
<tr>
<td>259.0 ± 15.8</td>
<td></td>
<td></td>
<td>280.5 ± 17.8</td>
<td>279.7 ± 19.8</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td>286.0 ± 21.3</td>
<td>284.0 ± 13.7</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>13</td>
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<td></td>
<td>300.7 ± 15.8</td>
<td>281.2 ± 11.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td>316.9 ± 13.4a</td>
<td>263.3 ± 9.9b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td>353.7 ± 20.0a</td>
<td>296.4 ± 9.7b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
<td>385.8 ± 13.6b</td>
<td>291.6 ± 10.3c</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. The number of rats is indicated in parentheses. Means in a row with unlike superscripts are significantly different (P < 0.05).

### Table 2

**Effect of maternal malnutrition and α-methyl-p-tyrosine (αMT) administration on body and brain weights of rat pups**

<table>
<thead>
<tr>
<th></th>
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<th>Normal αMT</th>
<th>Malnourished</th>
<th>Malnourished αMT</th>
<th>2-way ANOVA</th>
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</thead>
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<td>Diet</td>
<td>αMT</td>
<td>Diet</td>
<td>αMT</td>
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<td></td>
<td>P</td>
<td>P</td>
<td>P</td>
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<td>Body weight</td>
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<td>Day 1</td>
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<tr>
<td>6.7 ± 0.2a</td>
<td></td>
<td></td>
<td>5.7 ± 0.2b</td>
<td>5.5 ± 0.1b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(10)</td>
<td></td>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td></td>
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<tr>
<td>Day 45</td>
<td></td>
<td></td>
<td>135.5 ± 4.7</td>
<td>139.2 ± 6.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>144.4 ± 5.6</td>
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<td>(13)</td>
<td>(13)</td>
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<td>149.6 ± 4.2</td>
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<td>Brain weight</td>
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<tr>
<td>Day 1</td>
<td></td>
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<tr>
<td>197.5 ± 4.4a</td>
<td></td>
<td></td>
<td>160.0 ± 2.9c</td>
<td>180.7 ± 5.4b</td>
<td>&lt;0.001</td>
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<td>(10)</td>
<td></td>
<td></td>
<td>(10)</td>
<td>(10)</td>
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<td></td>
<td></td>
<td>1158.5 ± 20.4b</td>
<td>1125.7 ± 18.8b</td>
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<td>1260.3 ± 7.8a</td>
<td></td>
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<td>(13)</td>
<td>(13)</td>
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<tr>
<td>1244.7 ± 12.9a</td>
<td>(9)</td>
<td></td>
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</table>

1 Values are means ± SEM. The number of rats is indicated in parentheses. Means in a row with unlike superscripts are significantly different (P < 0.05).
ture, prenatal malnutrition can result in long-lasting brain weight deficit through a mechanism involving losses of neurons, glia and myelin, as well as impaired dendritic differentiation (Levitsky and Strupp 1995, Resnick and Morgane 1983). The participation of postnatal malnutrition, as an additive component, in the long-term brain weight deficit observed could be eliminated because at this age no differences in body weight were found between malnourished and normal rats.

It has recently been reported that normally fed rats receiving 50% of the amount of food consumed by controls at a fixed time of day, showed a phase advance of the diurnal peak of circulating corticosterone (Challet et al. 1997), a hormone that has been shown to participate in controlling cellular division and maturation in the normal rat brain (Trejo et al. 1995). For this reason, the possibility that the timed administration of food used in this study could constitute a biological signal leading to changes in plasma corticosterone cannot be eliminated as a factor influencing brain development of prenatally malnourished pups. Nevertheless, it has been observed that the absence of maternal glucocorticoids during gestation caused a marked increase of cellular density and lower cellular maturation in the cerebral cortex of well-nourished fetuses. In contrast, enhancement of maternal glucocorticoids caused an advance in developmental parameters such as cellular density, maturation and synapses formation in relation to controls (Trejo et al. 1995). In light of data indicating that protein malnutrition during the perinatal period leads to increased basal plasma corticosteroid levels (Adlard and Smart 1972), it remains unclear whether the neuroanatomical changes induced by glucocorticoids in the cerebral cortex of normal fetuses can also occur in the brain of malnourished pups.

Measurements of NA concentration performed on d 1 of postnatal life revealed that the malnourished group had enhanced whole-brain NA concentration compared with normal rats. A similar but not significant trend was observed for visual cortex NA concentration. This result is consistent with previous reports showing increases of central NA concentration in models of prenatal malnutrition other than this one (Morgane et al. 1978). The mechanism by which prenatal malnutrition enhances brain NA concentration is poorly understood. In models of postnatal malnutrition, it has been found that nutritional injuries during lactation result in increased availability of brain tyrosine (Wurtman et al. 1974) and in enhanced activity of tyrosine hydroxylase (Marichich et al. 1979, Shoemaker and Wurtman 1971). However, because neurons storing NA and both tyrosine hydroxylase and dopamine-β-hydroxylase activities are present in the brain of fetal rats by the second half of gestation (Coyle and Axelrod 1972a and 1972b, Hamon and Bourgoin 1981) it is possible that a prenatal malnutrition-induced increase in central NA levels could be due to increased synthesis of this neurotransmitter, as seems to occur in postnatal malnutrition. Measurements carried out on d 45 of postnatal life showed no differences in whole-brain and visual cortex NA concentration between malnourished and normal rats. Interestingly, it has been found that brain NA concentration remains elevated during adulthood when malnutrition is continued through postnatal life (Morgane et al. 1978). Taken together, these results suggest that adequate nutrition during lactation and the postweaning period could normalize NA metabolism in the brain of prenatally malnourished rats. Nevertheless, it must be stressed that other monoamines that are affected by in utero malnutrition, (e.g. serotonin) appear to be insensitive to postnatal nutritional rehabilitation (Miller and Resnick 1980). α-Methyl-p-tyrosine

### TABLE 3

Effect of maternal malnutrition and α-methyl-p-tyrosine (αMT) administration on rat pups’ central noradrenaline concentration and release

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Normal AMT</th>
<th>Malnourished</th>
<th>Malnourished αMT</th>
<th>2-way ANOVA</th>
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<tbody>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Diet</td>
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<tr>
<td>Whole brain concentration</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>439.9 ± 10.8b</td>
<td>347.8 ± 31.2c</td>
<td>555.9 ± 31.7a</td>
<td>429.3 ± 9.6b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Day 45</td>
<td>1644.7 ± 132.8a</td>
<td>1211.9 ± 49.8b</td>
<td>1755.6 ± 106.3a</td>
<td>1927.9 ± 119.4a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Visual cortex concentration</td>
<td>294.1 ± 24.4ab</td>
<td>177.8 ± 17.2c</td>
<td>349.4 ± 16.5a</td>
<td>248.3 ± 31.1b</td>
<td>&lt;0.025</td>
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<tr>
<td>Day 45</td>
<td>760.0 ± 33.8</td>
<td>745.8 ± 35.5</td>
<td>755.2 ± 66.3</td>
<td>823.9 ± 32.2</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Visual cortex release</td>
<td>5.9 ± 0.9b</td>
<td>3.9 ± 0.7b</td>
<td>11.5 ± 0.7a</td>
<td>6.7 ± 1.4b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Day 45</td>
<td>7.8 ± 0.9b</td>
<td>14.2 ± 1.1a</td>
<td>6.9 ± 1.2b</td>
<td>10.6 ± 1.7b</td>
<td>&gt;0.05</td>
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1 Values are means ± SEM. The number of rats is indicated in parentheses. Means in a row with unlike superscripts are significantly different (P < 0.05).
FIGURE 1 Effect of maternal malnutrition and \(\alpha\)-methyl-\(\beta\)-tyrosine (\(\alpha\)MT) administration on the extent of the cortical area (dark areas) occupied by high energy transcallosal evoked responses (TER) and on the peak-to-peak amplitude of TER. Values are means \(\pm\) SEM. The number of rats is indicated in parentheses. For TER area, two-way ANOVA indicated significant effects of diet \((P < 0.025)\) and \(\alpha\)MT \((P < 0.005)\), as well as a significant interaction diet \(\times\) \(\alpha\)MT \((P < 0.001)\). For TER amplitude, two-way ANOVA indicated a significant effect of diet \((P < 0.005)\) but not of \(\alpha\)MT \((P > 0.1)\), as well as a significant interaction diet \(\times\) \(\alpha\)MT \((P < 0.005)\). Means for each variable with unlike superscripts are significantly different \((P < 0.05)\).

treatment to normal and malnourished dams during the last 2 wk of pregnancy reduced both whole-brain and visual cortex NA concentration in the offspring. \(\alpha\)MT reduces noradrenaline synthesis by inhibiting tyrosine hydroxylase, the rate-limiting factor controlling brain catecholamine synthesis. Reductions of 30–50% in NA levels have been found in the brains of rats that were subjected to a diet containing 0.3% \(\alpha\)MT (Johnson et al. 1967). In this study, such a pharmacological treatment chronically administered to malnourished pregnant rats resulted in a 22.8% reduction of the NA concentration in the brain of newborns and in a 28.9% reduction of the NA concentration in the visual cortex of pups, leading to central NA concentrations roughly comparable to those observed in pups born from well-nourished dams. The foregoing biochemical data indicate that the pharmacological treatment was effective in reducing the central NA concentration in the malnourished-\(\alpha\)MT group even though food, including the drug, was consumed soon after presentation. In this respect it has been shown that a single acute dose of \(\alpha\)MT in rats decreases brain stem NA concentration for \(\geq 3\) d (Xiao et al. 1995). Consequently, in this study, it can be assumed that ingestion of \(\alpha\)MT once a day was sufficient to maintain stable low levels of brain NA throughout the pharmacological treatment. Moreover, a comparable reduction of central NA concentration was observed in the normal-\(\alpha\)MT group having free access to food.

Determinations of visual cortex NA release showed that at d 1 of postnatal life, malnourished newborns had higher NA release than normal pups. Although the mechanism by which malnutrition may alter the release of central NA is unclear, it could be related to metabolic and endocrine factors that increase brain NA synthesis (Marichich et al. 1979, Shoemaker and Wurtman 1971, Wurtman et al. 1974). Nevertheless, mechanisms other than increased NA synthesis, such as altered development of the \(\alpha\)-2 noradrenergic system, also could account for the increased NA release. In fact, it has been pointed out that development of \(\alpha\)-adrenergic receptor systems in rats depends upon maturation of their presynaptic nerve terminals (Deskin et al. 1981). In light of this observation and keeping in mind that the noradrenergic system is “operative” by the end of gestation, it is possible that metabolic changes induced by malnutrition could alter the maturation of central NA terminals, thereby impairing the development of the \(\alpha\)-2 noradrenergic system. Thus the impairment of NA regulatory mechanisms in the brain together with the enhanced central NA synthesis could lead to increased release of NA. In this respect, in cortical slices from rats subjected to early postnatal malnutrition, it has been shown that the \(\alpha\)-2 receptor agonist clonidine was unable to depress NA release, indicating a disruption of \(\alpha\)-2 regulatory mechanisms (Belmar et al. 1996). Because pups born to malnourished dams receiving \(\alpha\)MT treatment exhibited visual cortex NA release values roughly comparable to those observed in normal pups, it appears that the pharmacological treatment was able to depress the enhanced central NA synthesis and release, allowing for an apparently normal behavior of NA presynaptic terminals of the newborns. At 45 d of age, the normal-\(\alpha\)MT group exhibited slight but significant enhancements in visual cortex NA release compared with the other groups. This result suggests a long-term effect of the chronic prenatal \(\alpha\)MT treatment on \(\alpha\)-2 adrenoreceptor mechanisms, although, as far as we know, no data are available on the effect of such a pharmacological treatment on \(\alpha\)-2 adrenoreceptors.

Prenatal malnutrition induced a reduction in both the ex-
tent of the area occupied by high energy TER and the peak-to-peak amplitude of these responses. It is possible that these reductions could be due at least in part to an enhanced axonal pruning of callosal projections consequent to prenatal malnutrition-induced central noradrenergic hyperactivity. Indirect support for this alternative is found in studies showing that prenatal malnutrition decreases the size of the corpus callosum in rats (Zimmerberg and Mickus 1990). In contrast to malnourished animals, malnourished-αMT rats exhibited area and amplitude of high energy TER similar to those of normal rats. This indicates that pharmacological reduction of central NA prevented functional deficits of interhemispheric connectivity in the malnourished-αMT group, probably by restoring the normal trophic role of NA during the prenatal life and thereby decreasing excessive callosal pruning during perinatal life. Administration of αMT to normal pregnant rats affected neither the area nor the amplitude of high energy TER. This would indicate that moderate reduction of central NA in normal rats by the αMT treatment did not disrupt the functional callosal connectivity between the two visual areas. As pointed out in the literature (Maeda et al. 1974, Parnavelas and Blue 1982, Wendland et al. 1977), only severe reduction of central NA consequent to electrolytic lesion of the locus coeruleus or injection of 6-hydroxydopamine could alter the neuroanatomical development of the cerebral cortex of normal pups.

Prenatally malnourished rats exhibited lower interhemispheric asymmetry of VER than normal rats. Interhemispheric asymmetry of sensory cortical evoked responses appears to be a normal feature of humans (Beck and Dustman 1975), cats (Bianki and Filippova 1977) and rats (Soto-Moyano et al. 1989). In humans, VER asymmetry has been correlated to mental performance. In fact, diminished VER asymmetry has been found in children belonging to socioeconomically deprived populations (Beck and Dustman 1975), and losses of asymmetry of VER have been reported in Down’s syndrome (Beck and Dustman 1975). The mechanisms involved in the generation of electrophysiologic interhemispheric asymmetry are complex and not entirely elucidated, but could be related to anatomical and/or neurochemical lateralizations. For example, in rats the primary visual cortex is asymmetrical in volume, reflecting interhemispheric differences in the number of cells (Galaburda et al. 1986). Because asymmetry is found in the brains of human fetuses and newborn rats (Geschwind and Galaburda 1985), thus establishing the importance of intrauterine influences, it is plausible that prenatal malnutrition-induced central noradrenergic hyperactivity could impair the neural substrate underlying VER asymmetry by enhancing normal regressive processes. Administration of αMT to malnourished dams during pregnancy prevented the reduction of VER asymmetry in the offspring. In fact, no significant differences in this variable were found between normal and malnourished-αMT rats. This result supports the idea that an increased NA synthesis was involved in the reduction of VER asymmetry in the malnourished group. However, it is also true that αMT treatment on the one hand, reduces brain dopamine synthesis (Seiden et al. 1975), whereas the other hand, turnover of this neurotransmitter has been reported to be increased in the brain of rats subjected to perinatal malnutrition (Marichich et al. 1979). These two observations lead to the question of whether reduction of brain dopamine synthesis also participated in the effects observed. Because no data are presently available supporting a trophic role for dopamine on brain development, this point remains open for further investigation.

In summary, the present neurochemical and electrophysiologic evidence indicates that αMT treatment during fetal life prevents disorders induced by prenatal malnutrition on interhemispheric connectivity of visual areas and on interhemispheric bioelectrical asymmetry, probably through a mechanism that involves reduction of the enhanced activity of central NA and thereby restoration of the normal trophic role of this neurotransmitter during early stages of brain development.

LITERATURE CITED

MALNUTRITION, NORADRENALINE AND BRAIN DISORDERS


